

Mead and Other Fermented Beverages

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14.1 Introduction to Mead

14.1.1 Definition, Historical Aspects of Mead, and Perspectives

Mead is a traditional alcoholic beverage containing an alcoholic strength, by volume, between 8% and 18%, which results from the alcoholic fermentation of diluted honey by yeasts. It is a popular beverage in eastern Europe (Poland, Slovenia) and in the Baltic states, being also widely consumed in England, Germany, and, especially, in the African countries, for instance, Ethiopia and South Africa. In Portugal mead is still homemade, produced according to the traditional and empirical procedures. This alcoholic beverage is recognized as the oldest consumed by humans, perhaps even before wine, and probably the precursor of beer. It has a long heritage of use for over 5000 years, even though the available archaeological evidence for its production dates back to 7000 BC. The first batch of mead probably occurred when it rained into an open pot of honey and the wild yeast did the rest [1]. Pottery vessels containing mixtures of mead, rice, and other fruits with organic compounds of fermentation have been found in northern China; the first known description was found in the Rigveda and dates back to 1700 to 1100 BC [2]. The long tradition of mead consumption led to the coining of the term “honeymoon,” because, in addition to being drunk in great quantities at weddings, the newlyweds usually had the practice of drinking mead for one month (a moon) after the ceremony, with the belief that a child would be born 9 months later [3].

Although in the past its use was widespread, the development of civilizations and agricultural resources triggered the replacement of mead by other beverages, like wine and beer, in many areas of the world. In northern Europe, where vines are not cultivated, mead consumption was quite popular until wine was imported at a low cost from the

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southern regions. In the past few years there has been a huge spike in demand, after the drink became fashionable in America. The American Mead Makers Association, an organization dedicated to promoting mead and bringing together mead makers, lists almost 240 mead brewers in the United States and 40 in the rest of the world.

14.1.2 Mead Styles

Mead is an alcoholic beverage made by fermenting a mixture of honey and water. Depending on the proportion to which honey is diluted, at 1:0.5, 1:1, 1:2, or 1:3 (honey:water), different types of mead are obtained. Worts that contain a high concentration of sugar (1:0.5 or 1:1) are prepared in fed-batch, successively adding appropriate portions of honey to avoid premature fermentation arrest due to excessive osmotic pressure [4]. A weak or watered mead is called hydromel, and sack mead is a sweeter mead owing to the addition of honey [3]. Using a terminology similar to that used in wine, mead styles are classified as dry, semisweet, or sweet, according to its final sugar concentration [5].

To enhance its character and complexity, a variety of fruits, vegetables, herbs, or spices (ginger, cardamom, cloves, thyme, rosemary, bay leaves, sage, parsley, fennel, cinnamon, nutmeg, lemon or orange peels, among others) may be added to, during, or after fermentation. Traditional mead or show mead is made using either honey from a particular flower source or a multifloral honey. Show mead is produced only by honey's fermentation; nutrients and additives are tolerable but additional spices, fruits, or herbs are not allowed. Regarding traditional mead, small amounts of spices, fruits, or herbs are permitted without ever overpowering the honey flavor or aroma [6].

According to the American Mead Makers Association (www.meadmakers.org) there are several mead styles depending on local traditions and specific recipes. Pyments, cysers, and melomels are types of mead that include the addition of fruit or fruit juices. Pymment is a fermented beverage made from a mixture of grape juice and honey or from a blend of grape wine and mead after fermentation. It has a distinct grape wine character, manifested in acidity, tannin, and other grape characteristics, but the honey character should balance the fruity flavors. Cyser or apple honey cider is made from a mixture of honey and apple juice or cider without additional water. This beverage has a distinct apple character with a pronounced honey aroma, sweet and similar to a sherry. Concerning melomels, these are meads that contain one or a blend of fruits, which contribute with subtle acidic notes to intense, instantly recognizable fruit flavors. Metheglin is mead made with spices and/or herbs, and rhodomel is made from honey and rose petals. Another alcoholic beverage that can be made from honey is braggot, a type of beer made with a mixture of honey and malt, characterized by an aroma of honey and malt, with some bitterness due to the hops. Also, brandies and spirits can be produced from distilling mead. In addition, a sparkling beverage with high amounts of carbon dioxide resulting from a second natural fermentation either in bottle or in tanks may be produced [3].

14.1.3 Mead Product Derivatives

Honey is produced practically throughout the whole world, 90% of which is consumed as table honey and 10% is distributed among the food, cosmetic, and pharmaceutical industries. Different new products may be produced from mead, such as vinegar. In fact, by performing the acetic fermentation of mead, Ilha et al. [7] obtained approximately 5 L of honey vinegar with 90 g/L of acetic acid, using 1 kg of bee honey as raw material. This honey vinegar showed an acceptability index over 70% for appearance, color, odor, and flavor, indicating its good consumer acceptability. In South Africa it is possible to find in a meadery, the Makana Meadery, www.iquhilika.co.za, honey mead mustard made by mixing whole grain black mustard with freshly ground yellow mustard and mead vinegar, honey, and salt, resulting in a product with a complex flavor.

14.2 Characterization of Honey

Honey is “the natural sweet substance produced by honey bees from the nectar of plants (blossom honey or nectar honey) or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants (honeydew honey), which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature” [8]. For a long time in human history, it was an important source of carbohydrates and the only largely available natural sweetener [9,10]. In addition to its nutritional properties, honey is one of the products most referred to in old traditional medicine, because of its therapeutic potential in treating respiratory and gastrointestinal illnesses, in healing wounds and burns, and as an antimicrobial agent, among other biological proprieties [11,12].

According to its botanical origin, honey can be classified as monofloral or multifloral, if the bees forage predominantly on one type of plant or several botanical species, respectively [13].

Honey is mainly composed of carbohydrates, lesser amounts of water, and minor components [14] such as minerals, proteins, vitamins, lipids, organic acids, amino acids, phenolic compounds, enzymes, and other phytochemicals [15–17]. Nevertheless, honey composition is rather variable and dependent on the floral source, climate, environmental and seasonal conditions, as well as the handling and processing practices [11,13,18–24].

14.2.1 Carbohydrates

Carbohydrates account for about 95–99% of the dry matter in honey [14,25]. Fructose (38.2%, mean value) and glucose (mean value of 31.3%) are the major carbohydrates in honey, followed by sucrose (mean value of 0.7%) [9,14]. Additionally, 25 other oligosaccharides have been detected [9,14], including maltose, isomaltose, trehalose, turanose; trisaccharides erlose, raffinose, and melezitose; and trace amounts of tetra- and

pentasaccharides, among others [9,18]. According to the Codex Alimentarius [8], the minimum concentration of the reducing sugars, glucose and fructose, is 60% (w/w). The ratio of fructose to glucose is highly dependent on the nectar source [18] and is usually 1.2:1 [26]. The concentration of these sugars influences the sweetness and texture of honey: fructose is sweeter than glucose and honeys with higher ratios of fructose/glucose remain liquid for longer periods because glucose is less water soluble than fructose [17,26].

14.2.2 Water

Water is the second most important component of honey, ranging between 15% and 20%, with an average value of 17.2% [9]. The water content of honey depends on several factors: climate conditions, degree of maturity of the hive, and treatments applied during nectar and honey collection and storage [17,25]. This parameter will influence its physical properties such as the viscosity [25]. Honey with a high water content usually presents preservation and storage problems because it increases the probability of product fermentation [25]. In fact, low water content contributes to the stability of honey, preventing fermentation and crystallization during storage [24].

14.2.3 Minerals

Minerals come from the soil and plants and are present in small amounts ranging from 0.04%, in the clear honeys, to 0.2%, in some dark honeys [18,27]. In addition, other elements may be added during the processes of centrifugation and storage [28]. Potassium is the major mineral, with an average of about one-third of the total [18,25,29–31], followed by calcium, sodium, phosphorus, magnesium, iron, manganese, and copper [9,25]. Trace elements like aluminum, iodine, chloride, fluorine, bromine, and barium, among others, are also present in honey [14,32]. The mineral composition depends on the environment, geographic location, and botanical species [18,29,32,33]. In fact, honeys from light blossoms commonly have lower mineral content than dark honeys such as honeydew, chestnut, and heather [29].

14.2.4 Organic Acids

Organic acids comprise gluconic acid, resulting from the oxidation of glucose by glucose oxidase [14,25], followed in minor concentrations by pyruvic, malic, citric, succinic, and fumaric acids [14]. These acids account for 0.5% of the dry matter [9,25], for the acidity, and for the characteristic taste of honey [18]. Honey acidity is also dependent on the botanical species [24] and time of harvest [26]. The presence of osmophilic yeasts adapted to high osmotic pressures, such as high sugar concentrations, may be responsible for the increase in acidity [26]. So, low acidity, below the maximum limit of 50 mmol/kg, indicates the absence of undesirable fermentation [17]. Most honeys are acidic, with pH ranging from 3.4 to 6.1, and an average value of 3.9 [14,34]. However, this

parameter is not directly related to the free acidity owing to the buffering capacity of honey [26], which is dependent on phosphates, carbonates, and other minerals of honey.

14.2.5 Nitrogen Compounds

Amino acids, peptides, proteins, and nucleic acid derivatives are the major nitrogenous substances in honey. The amino acid content corresponds to about 10 g/kg [32]. The amino acid composition of honey is highly variable depending on its origin, thus the amino acid profile is a good indicator of the botanical and geographical origin of honey [18,22,32]. Proline is the major amino acid in honey, corresponding to values between 50% and 85% of total free amino acids [18,32]. Proline content should be above 200 mg/kg; values below 180 mg/kg indicate potential adulteration of the honey by sugar addition [14]. In addition to proline, 26 other amino acids have been identified in honey: glutamic acid, aspartic acid, glutamine, histidine, glycine, arginine, tryptophan, and cysteine, among others [18,32]. The protein content is relatively low, approximately 2 to 4 g/kg [14]. Proteins in honey are mainly enzymes: invertase, diastase, glucose oxidase, catalase [18], α -glucosidase, β -glucosidase [35]. Some enzymes come from the bees during the process of honey ripening [14]. The enzymes diastase and invertase are important for assessing honey quality, because they are used as indicators of honey freshness. Diastase catalyzes the hydrolysis of starch into disaccharides and monosaccharides and it is relatively stable to heat and storage, and invertase catalyzes the hydrolysis of sucrose to glucose and fructose. Hydrogen peroxide, H_2O_2 , the antibacterial factor found in honey, is regulated by the enzymes glucose oxidase and catalase. Thus, the enzymatic activity may indicate exposure to heat during processing and storage of the honey [14].

14.2.6 Vitamins

The vitamin content in honey is low and varies with the floral origin [36]. Most are water-soluble vitamins owing to the aqueous nature of honey and a low percentage of lipids [37]. Vitamins C (ascorbic acid), B_1 (thiamine), B_2 (riboflavin), B_6 (pyridoxine), B_3 (niacin), B_5 (pantothenic acid), and K (phyllochinon) have been reported in honey [9,25,32,37]. Ascorbic acid is the main vitamin found in honey, with concentrations ranging from 22 to 25 mg/kg [9] and it is found in almost all honeys. On the other hand, Ciulu et al. [36] observed a marked association between the concentration of vitamins B_3 and B_5 and the botanical origin of the product.

14.2.7 Phenolic Compounds

Honey contains a diversity of phenolic compounds as secondary constituents, such as flavonoids, phenolic acids, and phenolic acid derivatives. The main polyphenols are the flavonoids, in concentrations that can vary between 0.6 and 4.6 g/kg, and are mainly found in honey produced under dry and high-temperature conditions [9]. The flavonoids

present in honey are essentially flavanones and flavones, namely myricetin, tricetin, quercetin, hesperetin, lutein, kaempferol, pinocembrin, chrysin, pinobanksin, genkwanin, galangin, apigenin, and naringenin [9,15,18,19,21,38,39]. The phenolic acids are found in concentrations ranging from 0.01 to 10 mg/kg [18]. The predominant phenolic acids are gallic and *p*-coumaric, with caffeic, ferulic, chlorogenic, ellagic, syringic, vanillic, *p*-hydroxybenzoic, and cinnamic acids being minor constituents [15,21,38,40].

The composition in flavonoids of some honeys, such as heather, citrus, or chestnut, can be used for determining its botanical origin [40,41]. Dark-colored honeys contain more phenolic acid derivatives but fewer flavonoids than light-colored ones [14]. In fact, heather honeys are characterized by high concentrations of benzoic, phenylacetic, mandelic, and β -phenyllactic acids [18]. Considerable differences in composition and concentration of phenolic among monofloral honeys have also been found [14]. For instance, hesperetin proved to be a useful marker for the floral origin of citrus honey; kaempferol is a marker for rosemary honey, abscisic acid for heather honey, and homogentisic acid for strawberry tree (*Arbutus unedo*) honey [40].

The phenolic content of honey is highly related to its bioactive properties, namely antioxidant and antimicrobial activities. The antioxidant activity of honey has been reported by numerous authors [11,24,42–47]. Others have provided evidence of antibacterial activity of honey against pathogenic bacteria resistant to antibiotics [48–55] and against food spoilage bacteria [56].

14.2.8 Volatile Compounds

Volatile compounds of honey are derived from the botanical species or nectar source, from the transformation process carried out by bees, from heating or handling during processing and storage, or from microbial and environmental contamination [14,57,58].

Aroma compounds are present at very low concentrations, mainly as complex mixtures of volatile components with different functionality and relatively low molecular weight [59]. Indeed, more than 300 volatile compounds have been identified in different honeys, including hydrocarbons, aldehydes, alcohols, ketones, acids, esters, benzene derivatives, furans and pyrans, norisoprenoids, terpenes, and sulfur compounds [58,60,61].

Usually, monofloral honeys possess highly individual aroma profiles compared to multifloral ones [62]. The volatile profile represents a chemical fingerprint of monofloral honey because the nature and amount of volatile compounds are related to the floral source [41,63,64]. So, the determination of volatile compounds has been used to differentiate honeys according to botanical origin [57,61,63–65] and geographical origin [65–67]. The differences between the geographic sources can be attributed to climatic conditions and to the surrounding flora; nevertheless, the volatile compounds seem to contribute more to the differentiation of honey according to botanical origin than to country of origin [68]. In fact, a considerable number of volatiles have been suggested as possible markers of the following monofloral honeys: acacia, chestnut, eucalyptus,

heather, lime, and sunflower [69]; strawberry tree [70]; thyme [71]; and citrus, eucalyptus, and lavender [41,61]. For example, carvacrol and α -terpinene seem to be important in tilia honey, α -pinene and 3-methyl-2-butanol in sunflower, and *cis*-linalool oxide in acacia honey [68].

14.2.9 Color

The determination of honey color is a useful classification criterion for monofloral honeys, because it is related to the contents of phenolics, flavonoids, and minerals [15,21,32,72]. The mineral content influences the color and the taste; honeys with higher quantity of minerals have darker color and stronger taste [33]. The color of honey also depends on the processing, temperature, and/or time of storage [25] and can range from white-water, extrawhite, white, extraclear amber, light amber, amber, to dark amber [15]. However, it is important to ascertain that the color's intensity increases during storage owing to Maillard reactions, caramelization of fructose, and reactions with polyphenolic compounds [15,73].

14.2.10 The Natural Microbiota of Honey

The microbial population of honey includes microorganisms that come from the environment, soil, plants, and pollen and those that usually colonize the digestive tract of bees (primary sources of contamination) [25,74,75]. Thus, the microbial population of honey includes fungi (yeasts and molds) and spore-forming bacteria [74,75]. The intestine of bees contains high numbers of gram-positive bacteria (*Bacillus*, *Bacteridium*, *Streptococcus*, and *Clostridium* spp.) and gram-negative bacteria (*Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas*) and lower numbers of yeasts [76]. Additionally, microbial contamination may also have an origin in secondary sources, such as human handling, containers and equipment, insects, animals, and water [74,75]. Possible routes of microbial contamination include air (during packaging), handlers (from skin infections and fecal contamination), cross-contamination (from animals or animal products), and equipment (including residues of food and water) [75]. Whereas primary sources of contamination are very difficult to control, the secondary sources can be controlled with proper hygiene and good manufacturing practices.

The survival of microorganisms is influenced by honey's chemical composition, particularly by the low water content. Indeed, this parameter hampers microbial growth, especially of bacteria, which are generally less tolerant to high osmotic pressure, compared to fungi [25]. Also, the low pH and high sugar content play key roles in the survival and growth of microorganisms [14,34,76].

Even though bacteria can survive in this natural product, they are unlikely to replicate [75]. As a consequence, the detection of high numbers of vegetative bacteria might be indicative of recent contamination by a secondary source [34]. The consumption of honey contaminated with *Clostridium botulinum* spores is especially dangerous for

infants and children, with many reported cases of infant botulism. Although honey itself does not contain the toxin, the spores can theoretically build the toxin after digestion in infants until 1 year old [14].

Molds, or filamentous fungi, normally associated with honey include the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Mucor* [74,77]. These microorganisms can survive but do not tend to grow in honey [75]. The species *Bettsya alvei*, *Ascosphaera apis*, and *Ascosphaera major* may indicate recent contamination by inadequate bee hive management practices [17].

Honey naturally contains various osmotolerant/osmophilic yeasts that grow at low pH values and are not inhibited by high osmotic pressure. Most yeasts isolated from this environment include species of the genera *Saccharomyces*, *Debaryomyces*, *Hansenula*, *Lipomyces*, *Pichia*, *Schizosaccharomyces*, *Torula*, and *Zygosaccharomyces* [75]. Although studies on the quantification of yeast in honey are scarce, the values reported are normally low. In fact, less than 10 cfu of yeasts per gram was found in honeys of Central Argentina [17] and an average of 12 cfu/g in crude honey from India [78], whereas in honey from Brazil the number of yeasts varied from 5×10^2 [79] to 1.5×10^5 cfu/g [80].

Osmophilic or osmotolerant yeasts have the ability to convert honey's glucose and fructose into ethanol, carbon dioxide, and acids, making the product unsuitable for consumption. According to the literature, honey should meet the following quality criteria: maximum yeast count of 5×10^4 cfu/g and maximum glycerol content of 300 mg/kg; ethanol concentration should be less than 150 mg/kg [14]. Honey with moisture content less than 17.1% is safe from fermentation risk regardless of yeast count; however, a value above 20% means that the honey is always in danger of fermentation occurring [14].

14.3 Mead Production

The production of mead involves several steps that are presented in the diagram of Fig. 14.1.

Initially, honey is diluted with water in a proportion that depends on the type of mead desired. In most processes, honey must start at between 20 and 23°Bx [5,81–89]. Spices or herbs can be added, either as an extract or directly, prior to or during the process [3].

After dilution, a mixture of nutrients, nitrogen, minerals, and growth factors may be added if necessary, to stimulate yeast growth and fermentation. Also the adjustment of acidity may be done to obtain a better balance between sweetness and acidity. In general, the acids used for honey must adjustment are citric acid [4], malic acid [83–86], or tartaric acid [88,90]. A mixture of tartaric and malic acids may be used not only to adjust the acidity but also to increase the buffer capacity of honey must [82]. The must is subsequently sanitized, pasteurization being one of the most commonly used methods [82–86,89]. In contrast, other techniques are used with the aim of controlling or inactivating most wild microorganisms, including the addition of potassium metabisulfite

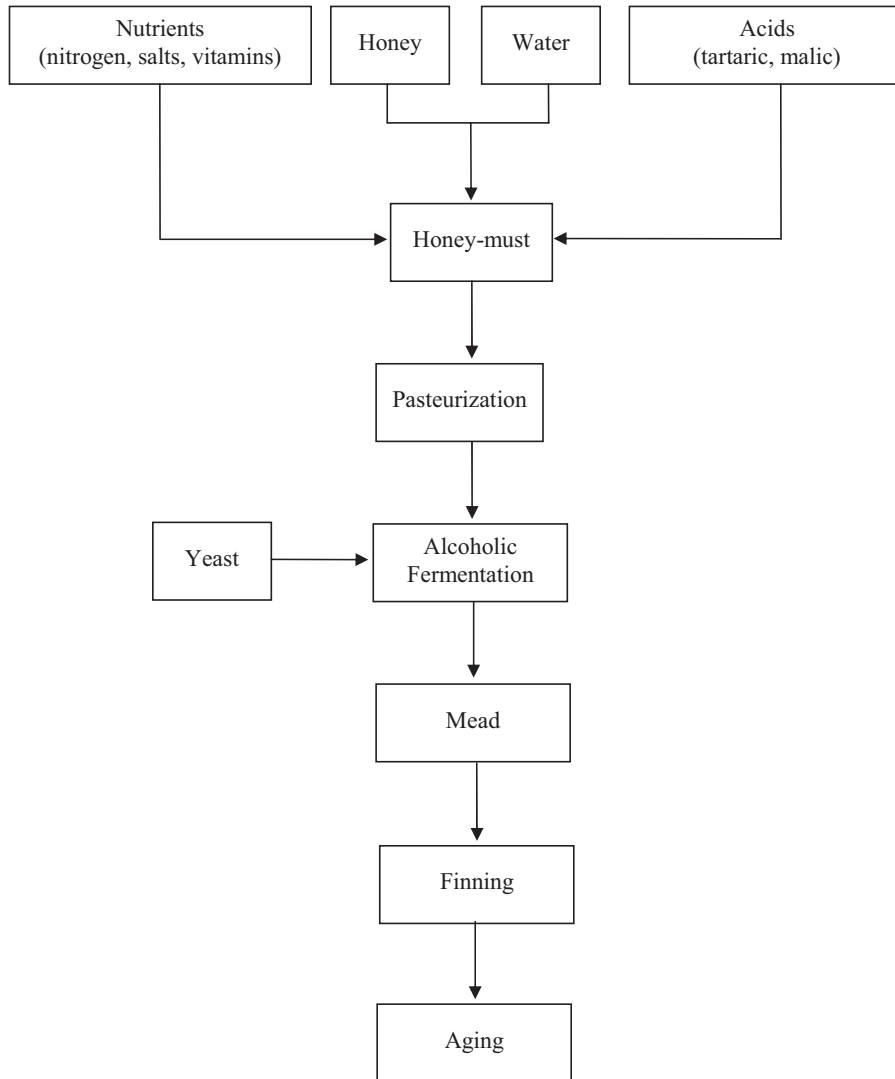


FIGURE 14.1 Diagram of mead production.

[88] and sulfur dioxide [90–92] or boiling of the must [4,92,93]. After the honey must treatment, it is inoculated with selected strains of *Saccharomyces cerevisiae* from culture collections or active dry yeasts available on the market. Fermentation is conducted at temperatures ranging from 22 to 25°C and is monitored daily to reduce the risk of premature fermentation arrest. The duration of fermentation depends on the type of honey, the nutrients added to the honey must, the size of the inoculum, and the fermentation conditions. After completion of alcoholic fermentation, the mead must be clarified by centrifugation or by using fining agents, such as bentonite, isinglass, egg

white, gelatin, and casein, and filtered before bottling. Fining agents are applied to obtain limpid and clear mead, eliminating substances in suspension as well as protein instability [94]. Aging is important in mead production, particularly for the development of favorable aroma compounds, generally moving from a harsh, acidic, unpleasant taste to a smooth to a mellow beverage with a nice bouquet and fragrance [3]. The length of aging can be from months to years, depending on the type of mead. In general, lighter meads will be ready sooner and darker, sweet meads and those with higher alcohol content will need more time to fully develop [3].

14.3.1 Control of Honey Must Fermentation

Saccharomyces cerevisiae metabolizes glucose and fructose through the Embden–Meyerhof pathway with the formation of 2 mol of pyruvate per mole of hexose. The pyruvate is decarboxylated by pyruvate decarboxylase to acetaldehyde, which is reduced to ethanol with the concomitant oxidation of NADH coenzyme formed in the oxidation of glyceraldehyde 3-phosphate to 1,3-diphosphoglyceric acid. The effective ethanol yield depends on the strain, as well as on the fermentative conditions, among which are the temperature and the must composition. In addition to ethanol, *S. cerevisiae* produces small amounts of glycerol, higher alcohols, diacetyl, acetoin, 2,3-butanediol, succinic acid, and traces of acetic acid, lactic acid, and acetaldehyde [95–97], which have a strong impact on final taste and aroma composition.

Despite the excellent properties of honey, mead production faces several problems, namely, slow or premature fermentation arrest, lack of uniformity of the final product, and production of yeast off-flavors. Many factors might be related to these problems, such as honey variety, medium composition (vitamin, minerals, and nitrogen content), fermentative yeast, and fermentation conditions (temperature and pH) [98].

The influence of the honey type has been evaluated in mead production [90]. Light honey, in comparison to dark, has a deficiency in the amount of nitrogen compounds and in the content of minerals that must be fulfilled by supplementation, taking into account the yeast's requirements.

14.3.1.1 Yeasts

The unpredictable nature of spontaneous fermentation and stock fermentation may be associated with the risk of undesirable flavor occurrence [81]. Hence, inoculation with selected yeasts is a common practice to control the nature and quantity of fermentation products, particularly metabolites that impair the final quality of the product. The yeasts used in mead production are usually strains of *S. cerevisiae* with suitable characteristics as required for wine and beer production: vigorous fermentative activity, tolerance to ethanol and sulfur dioxide, tolerance to temperature variation, and ability to flocculate easily after completion of fermentation. In a previous work, the fermentative abilities of five strains of *S. cerevisiae* isolated from Portuguese honey were compared to those of a commercial wine strain [90]. According to the results, the performance of the strains isolated from

honey was similar to that observed in those available on the market, which are used in the production of other beverages. Even though most mead is produced using commercial yeast strains, the traditional mead produced in some African countries still uses natural fermentation conducted by microorganisms initially present in the substrates and fermentation equipment. Fortunately, the yeasts *S. cerevisiae* [99] and *Kluyveromyces bulgaricus* [100] appear to be the dominant microorganisms in this traditional beverage.

In wine, mixed culture fermentation has been exploited to enhance aroma and flavor and to obtain different types and styles of the product [101]. This practice has been tested in mead production by Chen et al. [81], who successfully used multiple yeast inoculations with different strains.

The use of a reduced inoculum of *S. cerevisiae* can be associated with sluggish and stuck fermentations [102]. So, to provide evidence for this claim, Pereira et al. [83] studied the effect of the inoculum size on yeast fermentation performance, as well as on mead composition and the volatile compounds production. Increasing the pitching rate resulted in significant fermentation time saving, even though high inocula could lead to lower production of desirable aromatic compounds.

14.3.1.2 Nutrient Supplementation

The problems of honey must fermentation are considered to be due to a deficiency of nitrogen, minerals, and other growth factors [2]. The correction of these nutritional deficiencies may reduce stress sensitivity of the yeast, improving fermentation performance [103].

Vitamins, whose concentration is not usually limiting, are required by yeast cells for many enzymatic reactions [104,105]. Minerals are required as cofactors for several metabolic pathways influencing the rate of sugar conversion [106]. Nevertheless, nitrogen deficiency has been reported as the major cause of stuck or sluggish fermentation of grape juice [96,107], because nitrogen affects yeast growth, yeast fermentation rate, and fermentation length [108]. Nitrogen concentration also regulates the formation of by-products, such as H₂S, fatty acids, higher alcohols, and esters, among others, which affect the chemical and sensorial properties of the alcoholic beverage [96,109,110]. In alcoholic fermentation, *S. cerevisiae* normally requires a minimum of 267 mg/L, expressed as nitrogen, for complete fermentation of a must containing 200 g/L hexoses (glucose plus fructose), in an industrially reasonable time [111]. Despite this, there are differences in the nitrogen demand according to the industrial yeast strain or the quality of the nitrogen source or the must sugar concentration [112,113].

Taking into account all the knowledge used in wine production, honey must was optimized by supplementing it with potassium tartrate, malic acid, and diammonium phosphate (DAP) and fermentation time was reduced to 11 days [82]. Even under these improved conditions, the available sugars were not completely consumed by yeasts and a certain amount of residual assimilable nitrogen remained in all of the meads, even in controls to which no nitrogen was added, suggesting that other factors could account for the reduced yeast activity in honey must fermentation. In addition to reducing the

fermentation length and increasing specific growth rate of yeasts, the addition of DAP to honey must can contribute to the enhancement of the fruity character of mead [86].

In fact, the supplementation of nitrogen deficiencies with DAP addition is a widespread practice in mead production [5,7,83–85]. In other cases, the honey must nutritional deficiencies are supplemented in the form of commercial nutrients [89,90,93]. In the fermentation of longan mead, Chen et al. [81] found that the addition of commercial nutrients containing yeast hulls, yeast extract, DAP, vitamin B₁, magnesium sulfate, folic acid, niacin, and calcium pantothenate only attained high fermentation rates. Also using commercial nutrients, Gomes et al. [91] detected high sugar consumption and high production of ethanol, acetic acid, and glycerol with a concentration of 0.88 g/L.

There are references in literature to other natural supplements that can be added to mead to improve yeast growth or yeast fermentative activity: black rice, a natural nutrient for yeast, as a source of fungal glucoamylase [99,114]; fruit juices as a source of acids and growth factors [2]; or even pollen [88]. In this study, the addition of pollen improved fermentation rates, ethanol yield, and final sensory attributes.

14.3.2 Yeasts Immobilization in Mead Production

Whole-cell immobilization may be defined as the physical confinement or localization of intact cells to a certain defined region of space with the preservation of some desired catalytic activity [115]. Methods to immobilize microorganisms have gained attention in the past few decades and are being successfully applied in alcoholic beverage production. The use of these techniques has made it possible to reduce labor requirements, to simplify time-consuming procedures, and thereby to reduce costs [116].

So, to be attractive for industrial purposes the methodology must be robust, not susceptible to contamination, able to impart correct flavor changes to the beverage, not liable to cause oxidation of the product, and able to use commercially acceptable supports and organisms [116].

To obtain the desired product it is fundamental to select a suitable support for cell immobilization, and the choice depends on the process in which it will be applied as well as the process conditions [117]. Generally, four major categories of immobilization techniques can be distinguished, based on the physical mechanism employed: attachment or adsorption on solid carrier surfaces, entrapment within a porous matrix, self-aggregation by flocculation (natural) or with cross-linking agents (artificially induced), and cell containment behind barriers [117,118].

In comparison with free cells, the immobilization may induce alterations in cell growth, physiology, and metabolic activity and may affect their tolerance to stress factors and the formation of aroma compounds. Mass transfer limitations by diffusion, disturbances in the growth pattern, surface tension and osmotic pressure effects, reduced water activity, cell-to-cell communication, changes in the cell morphology, and altered membrane permeability are some factors considered responsible for alterations through immobilization [115].

Immobilized yeast cells have not been widely used in mead production. Indeed, only a few studies have been reported on this theme. To our knowledge, the pioneer work on

this matter was conducted by Qureshi and Tamhane [119] using whole cells of *S. cerevisiae* immobilized in calcium alginate gels to produce mead. The optimum pH for alcohol production was 4.5 or 5.5 when free or immobilized cells, respectively, were used. The authors produced mead for a period of more than 3 months using immobilized cells, thus reducing the problems of contamination and secondary fermentation associated with traditional mead production. Later, the same authors [87] used two series reactors separately packed with immobilized cells of *S. cerevisiae* and *Hansenula anomala* to produce meads of controlled quality and reduced the time period of production and eliminated the costlier aging process.

Navrátil et al. [93] used a two-column packed-bed system with an entrapped ethanol-tolerant distillery yeast of *S. cerevisiae* to provide higher ethanol productivity and thus to make the process more efficient. Yeast cells were immobilized in calcium pectate, which has higher mechanical stability than calcium alginate. The system enabled an increase in the fermentation rate and mead to be produced in a continuous mode.

The capacity of two sodium alginate concentrations, 2% and 4%, to immobilize *S. cerevisiae* yeast strains QA23 and ICV D47 in the context of mead production was investigated by Pereira et al. [84]. Neither of the alginate concentrations was able to prevent cell leakage from the beads. Even so, at the end of the fermentation, the number of cells entrapped in the beads was higher than the number of free cells, and the total 4% alginate bead wet weight was significantly higher than the 2% alginate bead wet weight. The fermentation length was 120 h for both yeast strains and the evaluation of mead quality showed that the yeast strain had significantly more influence on the physico-chemical characteristics than the alginate concentration.

To avoid cell leakage, the potential of application of immobilized yeast cells on single-layer Ca–alginate or double-layer alginate–chitosan was assessed for mead production [85]. Minor differences were detected in the fermentation length and in the rate between fermentations conducted with free or immobilized cells, even though higher concentrations of viable cells were achieved in immobilized systems. The double-layer alginate–chitosan had no advantage compared with the single-layer Ca–alginate, as the numbers of free cells in the medium resulting from cell leakage were similar. Meads obtained with entrapped yeast cells presented less ethanol and glycerol and more acetic acid, presenting larger amounts of volatile compounds. Immobilized cells produced meads with higher concentrations of fruity characteristic compounds such as ethyl octanoate and ethyl hexanoate; however, the concentrations of undesirable compounds, namely ethyl acetate and octanoic and hexanoic acids, in such meads were also higher.

14.4 Aroma of Mead

The aroma profile is one of the most typical features of a food product, for both its organoleptic quality and its authenticity [32]. The aroma of mead has contributions from honey, inoculated yeast, and technological processes [2,81,83,85,86].

14.4.1 Honey-Derived Volatiles

The honey quality, which is crucial in the consumer's assessment, is strongly dependent on the botanical and geographical origin of the product [9,58]. Honey aroma is very complex and involves several volatile compounds; however, not all have a significant impact on the aroma. In general, the impact of a given compound depends on the extent to which the concentration exceeds its odor threshold. It is important to state that some synergistic and/or antagonistic interactions between various components may occur, and thus, even compounds present in low concentrations may contribute to honey aroma [58,62]. To determine the influence of the volatile compounds on overall honey aroma, odor activity values (OAVs) should be assessed by dividing the concentration of each compound by its perception threshold. Only the compounds with OAVs greater than 1 (or near) may have contributed to the honey aroma [58]. The same volatile compounds identified in various honey samples can be characterized by a wide range of aroma descriptors, for example, from bitter, rancid, or fishy to sweet and flowery (Table 14.1).

Sensory evaluation, based mainly on attributes of aroma and taste, is one of the most useful tools in honey characterization [61,120,121]. Some of the aroma attributes proposed have been floral, fruity, candy, waxy, resin, wood, citric, acidic, spicy, balsamic, caramel, herbaceous, coffee/chocolate, cheese, chemical, and fermented, among others. The attributes sweet, acid, astringent, ripe fruit, toasty caramel, woody, and spicy have been selected for taste characterization. honeys from different geographical and botanic origins differ regarding their sensory profile. For instance, the attributes flowery, fruity, waxy, jaggery-like, chemical, and caramel notes were the major variables among honey samples from India [120]. Castro-Vázquez et al. [61] identified the volatile compounds and the sensory descriptors that are more representative of different monofloral honeys, namely, citrus, rosemary, eucalyptus, lavender, thyme, and heather. These authors verified that citrus honeys were characterized by higher amounts of linalool derivatives and by fresh fruit and citric aromas; eucalyptus honeys had hydroxyketones and *p*-cymene derivatives together with cheese and hay aromas; lavender honeys had mainly hexanal, nerolidol oxide, and coumarin and the sensorial attributes balsamic and aromatic herbs aromas; finally, heather honeys were characterized by high contents of benzene and phenolic compounds and ripe fruit and spicy aromas. Regarding chestnut honeys from Spain it was verified that the volatile composition and sensory profile are greatly influenced by the geographic origin, i.e., honeys from the Spanish northeast presented significantly higher concentrations of aldehydes, alcohols, lactones, and volatile phenols, which are associated with herbaceous, woody, and spicy notes; honeys from the northwest area showed superior levels of terpenes, esters, and some benzene derivatives, closely related to honey-like, floral, and fruity notes [121].

14.4.2 Fermentation Yeast-Derived Volatiles

During alcoholic fermentation, yeasts produce a range of compounds with strong sensorial importance to the quality of the final product. Fermentative compounds,

Table 14.1 Odor Descriptors of Some Volatile Compounds Found in Honeys

Volatile Compound	Odor Descriptors
Benzaldehyde	Bitter almond; fragrant; aromatic; sweet; marzipan
Benzene acetaldehyde	Harsh; green
γ -Butyrolactone	Woody; toasty; caramel
Carvacrol	Pungent; warm
<i>p</i> -Cymenene	Citrus; pine
β -Damascenone	Fruity; sweet; honey
Decanal	Strong; sweet; orange peel odor; citrus taste; soap; fat
Dimethyl disulfide	Vegetable; cabbage; putrid
Dimethyl sulfide	Cabbage; sulfuric; gasoline; sweet; honey; acrid; cooked vegetables
Dimethyl trisulfide	Powerful; fish; diffusive
Ethyl acetate	Ethereal; sharp; wine-brandy-like; reminiscent of pineapple
Ethyl butyrate	Sweet; fruity; pineapple
Furfural	Bread; almond; sweet; woody; fragrant; fruity; cherry
Heptanoic acid	Rancid; sour; sweet-like; fatty
Hexanol	Balsamic; aromatic herb
Hotrienol	Hyacinth; balsamic; aromatic herb
Isophorone and ketoisophorone	Spicy
Lilac aldehyde	Flowery; fresh
Linalool	Sweet; floral; lavender; refreshing; citrus; orange; forest; geranium
2-Methylbutanal	Sweet; musty; aldehydic
3-Methylbutanal	Sweet; musty; aldehydic
Nonanal	Citrus; fatty; floral; green
Nonanol	Green; sweet; oily
Oak lactone	Woody; toasty; caramel
Octanal	Fat; soap; lemon; green
Pantolactone	Woody; toasty; caramel
Phenylacetaldehyde	Sweet; honey-like
2-Phenylethyl acetate	Flowery; sweet; champagne
Sinensal	Sweet; orange
Spathulenol	Cheese; hay

C.E. Manyi-Loh, R.N. Ndip, A.M. Clarke, Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities, *International Journal of Molecular Sciences* 12 (2011) 9514–9532; V. Kaškonienė, P.R. Venskutonis, Floral markers in honey of various botanical and geographic origins: a review. *Comprehensive Reviews in Food Science and Food Safety* 9 (2010) 620–634.

resulting from the metabolic activity of yeasts, represent quantitatively the majority of volatile compounds in wines [122]; therefore these microorganisms play an important role in the development of wine aroma. Since 2005, some research has been conducted on volatile compounds formation during mead fermentation. The production of volatile compounds is affected by several factors, including the yeast strain [81,99], cell condition (free or immobilized) [85], and inoculum size [83], as well as by the fermentation conditions [89]. In addition, the type of honey [89,123,124], and the honey must composition/formulation [4,82,86,88] can also modulate the formation of volatile compounds.

The volatile compounds produced by yeasts are alcohols, organic acids, esters, volatile fatty acids, carbonyl compounds, and volatile phenols, among others.

14.4.2.1 Alcohols

Alcohols are secondary yeast metabolites and, from a quantitative point of view, are the most important group of volatile compounds produced by yeast during alcoholic fermentation of sugars [125,126], inclusive in mead production [82,83,85,86,88]. Alcohols include 2-methyl-1-propanol (isobutanol), 2-methyl-1-butanol, 3-methyl-1-butanol (isoamyl alcohol), and 2-phenylethanol (with pleasant rose-like aroma), among others [125,126]. The most predominant alcohol in some meads has been 3-methyl-1-butanol, in concentration ranging from 90 to 350 mg/L [81–83,85,86,88,89,99], above the odor threshold of 30 mg/L [127,128]. In comparison, lower concentrations of 3-methyl-1-butanol were found in Slovak and South African meads [123]. Other secondary predominant alcohols present in mead are 2-methyl-1-butanol, 2-methyl-1-propanol, 1-propanol, and 2-phenylethanol [82,83,85,86,88,89,99,124].

Generally, concentrations of alcohols in mead are below 300 mg/L. Excessive concentrations, above 400 mg/L, may have negative impacts on the aroma and flavor, resulting in a strong, pungent smell and taste [125]. Mendes-Ferreira et al. [82] verified an inverse correlation between higher alcohols and nitrogen levels in mead, and Roldán et al. [88] found an increase in alcohols with pollen addition to the honey must. Moreover, the immobilization of yeast cells of *S. cerevisiae* seems to enhance the production of 1-propanol [85].

14.4.2.2 Esters

Esters are derived from a reaction between organic or volatile fatty acids and ethanol (ethyl esters) or between acetic acid and higher alcohols (acetates), being largely responsible for the fruitiness of wine and fermented beverages, and therefore they play an important role in the sensory composition of the related young products [82,83,126]. Ethyl acetate is quantitatively the most important ester found in mead produced in Portugal [82,83,85,86], Slovakia and South Africa [123], Spain [88], Slovenia [124], and southwest Ethiopia [99]. Ethyl acetate is an ester compound with a solvent-like odor [129,130] and an odor threshold of 12.3 mg/L [131]. Other esters found in mead in minor amounts are isoamyl acetate, 2-phenylethyl acetate, ethyl butyrate, ethyl hexanoate, and ethyl octanoate. These esters have pear-drop aromas (isoamyl acetate); honey, fruity, flowery aromas (2-phenylethyl acetate); and fruity, sweet aromas (ethyl butyrate, ethyl hexanoate, and ethyl octanoate) [129]. However, high variability in the concentration of these compounds is observed between meads, probably because different commercial yeast strains can produce variable amounts [125].

Ester production by yeasts increases with nitrogen concentration [82], with the addition of nutrients to the honey must, like pollen [88], and in mead fermented with yeast cells immobilized in single-layer of alginate or double layer of alginate–chitosan [85].

14.4.2.3 Volatile Fatty Acids

Volatile fatty acids include a mixture of straight-chain fatty acids, resulting from β -oxidation of fatty acids, usually referred to as short-chain (C2–C4), medium-chain (C6–C10), long-chain (C12–C18), and a group of branched-chain fatty acids, from the metabolism of the amino acids [126]. Acetic acid is quantitatively and sensorily the most important volatile fatty acid produced during alcoholic fermentation, accounting for more than 90% of the total volatile acidity [129]. Acetic acid at elevated concentrations imparts a vinegar-like character and it becomes objectionable at concentrations of 0.7 to 1.1 g/L, the optimal concentration being between 0.2 and 0.7 g/L [125]. In mead, concentrations of acetic acid less than 0.6 g/L have been reported [4,85,90], although Švecová et al. [132] found concentrations above 1 g/L in Czech meads. Volatile acidity increases during fermentation mainly as a result of acetic acid synthesis. So, values ranging between 0.4 and 4 g/L have been reported in mead [82,83,85,86,88,89,123]. As the fatty chain length increases, the volatility decreases and the odor changes from sour to rancid and cheese [126], characteristic of the compounds hexanoic, octanoic, and decanoic acids. Generally, octanoic acid is the main fatty acid in mead, followed by hexanoic and decanoic acids [82,83,85,88]. This fatty acid has an odor threshold of 0.5 mg/L [133] and its amount in mead can vary from 0.1 [88] to 6 mg/L [82]. On the other hand, Sroka and Tuszyński [4] verified that decanoic acid was in higher amounts in mead than octanoic and dodecanoic acids, but all of them were at concentrations below 30 mg/L. The concentration of medium-chain fatty acids was higher in meads supplemented with nitrogen compared to nonsupplemented fermentations [82,86,88].

14.4.2.4 Carbonyl Compounds

Yeasts produce various carbonyl compounds from sugar metabolism, acetaldehyde being quantitatively the most important, constituting more than 90% of the total aldehydes in wines and other alcoholic fermented beverages [134]. It contributes with “bruised apple” and “nutty” characters, when present at sensorily detectable concentrations, but can also be a sign of wine oxidation [125,126]. This compound has been found in meads produced by *S. cerevisiae* in concentrations between 5 and 30 mg/L [83,85,86,88], always above its perception threshold of 0.5 mg/L [127]. However, considerably higher concentrations of this compound were obtained for mead produced by any other yeast species, *Saccharomyces bayanus*, either in lime mead (608 mg/L) or in chestnut mead (1370 mg/L) [124]. In addition, the concentration of this compound appears to be related to the must composition, increasing with the addition of pollen [88] and nitrogen [86].

14.4.2.5 Volatile Phenols

Volatile phenols have a relatively low detection threshold and are, therefore, easily detected owing to their pharmaceutical odor [125]. Although they can contribute positively to the aroma of some wines, they are better known as off-flavors such “Band-Aid,” “barnyard,” or “stable” [129]. The most important volatile phenols are the ethylphenols,

4-ethylguaiaicol and 4-ethylphenol, and the vinylphenols, 4-vinylguaiaicol and 4-vinylphenol. Higher concentrations of 4-vinylphenol than 4-vinylguaiaicol have been found in mead produced with a multifloral dark Portuguese honey, but in concentrations below their detection thresholds [83,85,86]. This production was not affected by the yeast strain. Also in buckwheat and soy mead 4-methylphenol was detected, but again, in concentrations below the odor threshold [89].

Because beverages can contain a very complex set of volatile compounds, to estimate the contribution of an individual compound to the overall aroma it is important to determine the OAV [135]. The volatile compounds with more influence on the mead aroma profile are the alcohols (3-methyl-1-butanol and 2-phenylethanol), esters (mainly ethyl acetate, isoamyl acetate, ethyl butyrate, ethyl hexanoate, and ethyl octanoate), medium-chain fatty acids (hexanoic, octanoic, and decanoic acids), and acetaldehyde [83,85,86,89]. Even though few studies are available on the OAVs in mead, the results show that they depend on the inoculum size, yeast cell immobilization, and nitrogen addition to fermentation, as well as on the yeast strain and honey used in the mead production.

14.5 Sensory Evaluation of Mead

Aroma volatile compounds play a key role in determining the quality of beverages because they are the primary contributors to aroma and produce an effect on sensory characteristics [136,137]. Two main types of methodologies are used for evaluation of the quality of food and beverages. The identification and quantification of aroma compounds, as an objective analysis technique, or subjective methods based on human assessment of the quality characteristics of the food [138]. Sensory analysis is indispensable for the assessment of food flavor characteristics to identify the significant sensory and quality contributors to food quality and consumer preference [139]. Overall, the more important sensory characteristics of beverages are the smell, the taste, and, to a lesser extent, the color [140], and their assessment is performed by a panel of experts or consumers. However, sensory perception is variable within individuals, the context of the consumer experience, and the chemical composition of the product [139].

Even though the identification and quantification of aroma compounds in mead produced under different conditions have been assessed [4,81–83,85,89,123,124], there is a lack of evidence regarding the sensory quality of the mead produced.

Koguchi et al. [114] produced mead with honey and black rice and performed the sensory test of the beverages, revealing that mead made from Chinese milk vetch honey was acceptable, while that produced using buckwheat honey was not very palatable. The sensory characteristics of mead produced with cassava (*Manihot esculenta*) floral honey under farm conditions in Nigeria was also assessed [92]. In this study, the expert test panel included nine food scientists, who commented freely on fresh and stored mead samples. The color and taste of the meads were generally acceptable and the

characteristic aftertaste bitterness of samples was taken as both a positive and a negative attribute.

Vidrih and Hribar [124] studied the sensory properties of three different types of mead produced from chestnut, lime, and honeydew varieties of honey. The trained panelists chose the chestnut honey solution as the best raw material for mead production, followed by the lime and honeydew honey solutions. After fermentation, honey was added to meads and panelists preferred meads with 80 g/L sugar over meads with 40 g/L reducing sugar (dry mead with no reducing sugar is rather flat in taste and poor in body) and gave the best scores to the chestnut and lime meads. In the chestnut mead the reducing sugars masked the bitterness of the raw honey and the fermentation process improved the bouquet of the lime mead.

In short, the results of mead sensory analysis indicate that high sugar content is an important requisite to mead consumers.

The establishment of correlations between instrumental measurements of specific attributes and sensory characteristics may lead to a better understanding of the relationship between volatile composition and sensory properties, which is important to assess the quality of the beverage [139]. In this context, Roldán et al. [88] evaluated the influence of pollen addition on the sensory characteristics of mead, namely on visual (turbidity and color), aroma (quality and intensity), and taste (quality and intensity) characters, and verified that the aroma quality appeared to be related to the volatile compounds. The aroma of the control mead was described as floral (associated with 2-phenylethanol) and vinegar-like acid (presence of 3-methylbutyric and hexanoic acids, ethyl acetate, and high total and volatile acidity), masking other aromas, which decreased the aroma quality. Mead with a high amount of pollen added was characterized by toasted, bitter almond, and honey scents that masked all other aromas, principally consistent with its high phenylacetaldehyde levels. Briefly, the pollen addition led to an increase in the volatile contents of meads, consequently improving its sensory profile.

14.6 Conclusions and Perspectives

Beekeeping is a dynamic activity in frank expansion in recent years. Even so, it is necessary to continue to valorize the honey and simultaneously find alternatives for the honey that cannot be commercialized. Thus, mead production may emerge as a great alternative for profit and to add value to honey not suitable for trading. Despite being a traditional alcoholic beverage made since ancient times, its production is still mostly empirical and homemade. The producers find several problems during fermentation, such as lack of uniformity in the final product, slow or premature fermentation arrest, and the production of off-flavors.

Owing to the lack of scientific progress in this area, research since 2005 has been dedicated to solving some of the problems associated with mead production.

Most research has been focused on the type of honey, the honey must supplementation, and the fermentation conditions. Nevertheless studies related to the development of new production processes are scarce. For instance, the continuous fermentation process may emerge as an alternative, because, although the batch system has been the most used, it shows some deficiencies, in particular those related to low concentrations of reagents and the slow process of final product purification. Also, the application of immobilized yeasts, a technique with great potential and widely used in other beverages production, is not fully optimized in mead production. A previous study demonstrates that the use of multiple strains has a positive effect on mead fermentation, so it would be interesting to explore even further the use of mixed cultures.

In the same way, much is still unknown about the sensory analysis of mead. The studies available revealed that the tasters usually prefer a sweeter beverage. So, the research should also be focused on improving the sensory quality of mead, for example, by the addition of honey, fruit juices, or pollen at different stages of fermentation. The sensory quality of mead could also be improved with the aging, such as in oak vats. Owing to the absence of research in this area, it would be important to evaluate the effect of the aging process on the development of the aroma compounds of the beverage. However, to ensure a stable product and the safety of the consumer it is imperative to assess the shelf life of mead.

In conclusion, mead production is an area with limited research studies, so we consider that all the strategies proposed may contribute to obtaining a high-quality alcoholic beverage.

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